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# Characterization of the Molt Stages in *Penaeus vannamei:* Setogenesis and Hemolymph Levels of Total Protein, Ecdysteroids, and Glucose

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**Abstract.** The molting cycle of *Penaeus vannamei* juveniles was characterized by distinct and predictable changes in the setae of pleopods. The molt pattern was diecdysic with a relatively short intermolt period (40%) and a long proecdysial period (>53%). The levels of both total protein and ecdysteroids increased in the hemolymph during proecdysis, whereas the level of hemolymph glucose was low at meteecdysis and proecdysis and maximal during anecdysis. As revealed by SDS-PAGE, the relative concentrations of two polypeptides (32 kD; 175 kD) changed during the molting cycle.

## Introduction

Molting in arthropods includes not only the act of ecdysis, but also new cuticle formation, apolysis, the immediate postecdysis, and tissue growth (Passano, 1960). This dynamic cycle has been divided into four phases in crustaceans: (1) meteecdysis (stages A, B), the period immediately following ecdysis; (2) anecdysis (stage C), a period of tissue growth and accumulation of food reserves; (3) proecdysis (stage D), a period of active morphological and physiological changes in preparation of the next molt; and (4) ecdysis (stage E), the shedding of the old cuticle (Drach, 1939).

Several methods are used to determine the molt stages of crustaceans. These methods include histological examination of the integument, measurement of the size of the gastroliths or the regenerating pereiopods, and determination of setal development on the appendages. Determination of molt stages by the state of setogenesis on the appendages is rapid and inflicts little harm to the animals, even after repeated sampling. Setogenesis is used as a criterion to stage a number of decapods, including the natantians (Scheer, 1960; Kamiguchi, 1968), anomurans (Kurup, 1964), and macrurans (Aiken, 1973). Among the penaeids, criteria for assessing molt stages are described for *Penaeus duorarum* (Schafer, 1968), *Penaeus merguiensis* (Longmuir, 1983), *Penaeus esculentus* (Smith and Dall, 1985), *Penaeus stylirostris* (Huner and Colvin, 1979; Robertson *et al.*, 1987), and *Penaeus setiferus* (Robertson *et al.*, 1987).

Molting is stimulated in Crustacea by one or more of a group of closely related steroid hormones, the ecdysteroids (Skinner, 1985). Hemolymph ecdysteroids during the molting cycle have been measured in only a few decapods by use of radioimmunoassay (RIA) (Andrieux *et al.*, 1976; Chang *et al.*, 1976; McCarthy and Skinner, 1977; Keller and Schmid, 1979; Stevenson *et al.*, 1979; Chang and Bruce, 1980; Charmantier-Daures and De-Reggi, 1980; Hopkins, 1983; Jegla *et al.*, 1983; Soumoff and Skinner, 1983). No determinations are reported for penaeid shrimp. In most cases, hemolymph ecdysteroid titers increase rapidly during proecdysis; however, precise patterns are species-specific (*e.g.*, Stevenson, *et al.*, 1979; Chang and Bruce, 1980). Other hemolymph parameters, such as the levels of glucose (Telford, 1968) and protein (Dall, 1974) also undergo cyclic changes that correlate with the molt stage.

This paper describes the molting cycle of *Penaeus vannamei*, an economically important shrimp in the mariculture industry of the southern United States. We have characterized molt stages based on setogenesis of the

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pleopods, determined hemolymph levels of protein, glucose, and ecdysteroids, and analyzed the hemolymph proteins by polyacrylamide gel electrophoresis (PAGE).

## Materials and Methods

### *Animals*

*P. vannamei* juveniles were purchased from Ocean Venture Inc. in Port Lavaca, Texas. They were held for two weeks in 250 gallon circular tanks for acclimation at 20–22°C and at a salinity of 28–30‰. Shrimp (11.5–13.0 cm total length) were maintained individually in plastic boxes (23 cm × 12.5 cm × 12 cm) and fed once daily with commercial maturation diet at a rate of 4% total body weight (Rangen Inc., Buhl, Idaho).

### *Setogenesis*

To measure setogenesis, the distal third of the pleopod was excised, floated in saline on a microscope slide, and observed at 100× with a compound microscope. Photomicrographs were prepared from pleopods mounted in saline. Setal development was observed at 6-h intervals for 2 days after ecdysis and then twice daily until the next molt.

### *Hemolymph measurements*

Forty μl of hemolymph were withdrawn through the arthropodial membrane of the fifth pereiopod using a chilled microsyringe. Hemolymph sampling was performed with minimal handling of animals and took <20 s. This brief period of handling precluded induction of an endocrine-dependent stress response that would influence the levels of hemolymph metabolites during the time of sampling. All sampling was performed 2–5 h after the onset of the photophase. Sampling of pleopods and of hemolymph at four-day intervals had no apparent effects on the animals. Results from both sexes were pooled since preliminary studies had indicated no sex-related differences in hemolymph metabolite levels.

Ecdysteroid titers were determined in the hemolymph by RIA. Ecdysone antiserum was a gift from Dr. W. E. Bollenbacher (University of North Carolina, Chapel Hill). 23,24 $^3H_2$(N) ecdysone (specific activity 58–60 Ci/m*M*) was purchased from New England Nuclear (Boston, MA); the ecdysone standard was purchased from Sigma Chemical Co. (St. Louis, MO). Ten μl of hemolymph were added to 150 μl methanol. After precipitation of protein for 15 minutes at 4°C, the sample was centrifuged for 5 minutes at 12,000 × *g*. The supernatant was collected and evaporated under nitrogen, and ecdysteroids were measured (Chang *et al.*, 1976).

Hemolymph total protein was measured using the Coomassie brilliant blue test (Bradford, 1976).

Hemolymph glucose was determined by the glucose oxidase procedure (Mark, 1959) using a commercial reagent kit (Sigma Chemical Co.).

### *Polyacrylamide gel electrophoresis (PAGE)*

Hemolymph proteins were analyzed by slab gel electrophoresis (Laemmli, 1970). Native PAGE (6%), was performed with hemolymph mixed with a sample buffer (0.125 *M* Tris-HCl, pH 6.8) containing 0.01% bromphenol blue as the tracking dye. SDS-PAGE (10–15% linear gradient) was performed with hemolymph incubated in sample buffer containing 2% SDS and 1% 2-mercaptoethanol for 15 minutes in a boiling water bath. Electrophoresis was run at 50 V in the stacking gel and 100 V at the resolving phase. Gels were stained with Coomassie blue R for total protein or with dithiooxamide for copper (Whittaker, 1959).

## Results

### *The molting cycle: setogenesis*

Setae are external outgrowths from appendages such as uropods, pleopods, and antennule scales. In *P. vannamei*, the degree of setal development was not identical on different appendages and in different regions of the same appendage. For example, if pleopod setae were in stage $D_0$, most of the setae on the antennule scales were still in late anecdysis ($C_3$). Similarly, when the setae on the proximal portion of the pleopod entered proecdysis, the setae on the distal half of the pleopods were still at anecdysis. To standardize the criteria for determining molt stages, setogenesis was based on changes in the setae on the distal third of the pleopods.

*Stage A (metecdysis).* Stage A lasted about one day (Table 1), and the newly molted animals were inactive and did not feed. The exoskeleton was soft, parchment-like, and uncalcified. The epidermis was transparent with little pigmentation. Setal lumens were filled with translucent fiber-like matrices (Fig. 1), and the epidermis near the setal base was less granular than that of later stages.

*Stage B (metecdysis).* The exoskeleton hardened, presumably due to deposition of calcium, and epidermal pigmentation increased. At this time, setal matrices appeared granular and began to retract from the setal lumens towards the bases of the setae. An internal cone (conical base) began to form in each seta during later Stage B.

*Stage C (anecdysis).* Calcification of the exoskeleton was completed. This stage occupied 10 to 15 days (35–40%) of the intermolt period (Table 1). Animals were maximally active at this stage and resumed feeding. Most of the setal lumens were clear of setal matrix at this time.

Table I

*Characteristics of the molting stages of juvenile* Penaeus vannamei

| Stage | Duration | Feeding/activity | Exoskeleton | Epidermis | Setal development |
|---|---|---|---|---|---|
| A | 18–28 h (1–2%) | none/weak | soft | transparent | granular matrix fills lumen |
| B | 23–40 h (3–4%) | none/restored | hardened | granular | granular matrix retracts; internal cone formation begins; setal organs become visible |
| $C_1$ | 1.5 days (5%) | restored/maximal | hard | granular | granular matrix retraction completed, internal cone formation completed |
| $C_2$ | 6–8 days (20%) | maximal/maximal | hard | granular | |
| $C_3$ | 4–7 days (15%) | maximal/maximal | hard | granules very dense | |
| $D_0$ | 3–6 days (15%) | decreasing/maximal | No new cuticle yet | retracts (apolysis) | |
| $D_1$ | 8–10 days (28%) | decreasing/maximal | New cuticle appears | invaginates | new setae begin to develop |
| $D_2$ | 2–3 days (6–7%) | | Space forms between old and new cuticle | invaginates | new setae form barbules |
| $D_3$ | 1–2 days (3–4%) | no feeding, water is absorbed | old skeleton soft | | old setal organs disappear, new setae fold |
| E | 1–2 min | no feeding, body expands | old cuticle is shed | | |

Formation of internal cones was completed in the $C_1$ stage (Fig. 3). Setal organs, cylindrical structures that give rise to the setae (see Aiken, 1973), became clearly visible in the $C_2$ stage (Fig. 4). The $C_3$ stage differed from the $C_2$ stage (Fig. 4) in that during $C_3$ the setal bases were more dense and the setal organs more distinctive (Fig. 5), presumably due to mobilization of granules in the epidermis just before proecdysis.

*Stage D (proecdysis).* Proecdysis lasted 15 to 19 days and could be divided into stages $D_0$–$D_3$. Early proecdysis began with apolysis (Jenkin, 1966), the separation of the endocuticle from the epidermis. In *P. vannamei,* the process began first in the posterior region in the endopodites (Fig. 6) and was accompanied by the resorption and presumed decalcification of the exoskeleton. New cuticle was not present at the $D_0$ stage. As the epidermis retracted, it invaginated ($D_{1'}$) (Fig. 7) at the setal bases as new cuticle was deposited ($D_{1''}$). At the late $D_{1''}$ stage, the new epidermis continued to invaginate and new setae began to develop and protrude from the new cuticle (Fig. 8). At the $D_2$ stage (Fig. 9), new setae formed barbules and the setal spines extended into the base of the former seta. The epidermal retraction continued and resulted in large empty spaces between the old and new exoskeletons ($D_3$). Setal organs were no longer evident as discrete organs. Presumably as a result of muscular contraction, the new setae folded, and disrupted the regular pattern of setal arrangement (Fig. 10). Immediately before ecdysis, late proecdysis was characterized by absorption of water, expansion of the body, muscular contraction/relaxation, and breakage of the intercalary sclerites in the abdominal dip.

*Stage E (ecdysis).* As the animal shed the exuvium, the invaginated setae everted. Ecdysis lasted for less than 2 minutes (<1%). Early post-molt animals usually did not feed, although some newly molted animals consumed the old exuviae, possibly to recover calcium and other cuticular components.

### *The molting cycle: hemolymph ecdysteroids and metabolites*

We measured levels of circulating ecdysteroids and metabolites during different stages of the molting cycle as determined by the criteria of setal formation and morphology described above.

Figure 11 shows the ecdysteroid levels in the hemolymph during each molt stage. Ecdysteroid titers were approximately 30 pg/$\mu$l during metecdysis (A and B). The titers dropped to a minimum of about 16 pg/$\mu$l at stage $C_2$, began to increase at $C_3$ and reached a maximum of approximately 200 pg/$\mu$l at $D_{1'}$. The increases in ecdysteroid titer correlated with the onset of proecdysis and the events related to new cuticle formation.

The pattern of hemolymph protein levels was similar to that of the ecdysteroids. Hemolymph protein levels were low during metecdysis (20 mg/ml) and anecdysis ($C_1$) and increased to a plateau (85–95 mg/ml) at the proecdysis ($D_3$) (Fig. 12).

The changes in levels of hemolymph glucose (Fig. 13) during the molting cycle did not resemble the S-pattern





**Figure 1.** Early metecdysis, stage A. Most of the setal lumens are filled with setal matrix (sm). The bases of setae (sb) are agranular and setal articulations (sa) are opaque. (100×), scale bar = 45 μm.

**Figure 2.** Metecdysis, stage B. The setal matrix (sm) has begun to retract towards the base of the seta (sb), revealing the setal lumen (sl). (100×), scale bar = 45 μm.

**Figure 3.** Anecdysis, stage $C_1$. Retraction of setal matrix (sm) has neared completion and the setal lumens (sl) are almost empty. Formation of internal cones (ic) has been completed in most setae. (100×), scale bar = 45 μm.

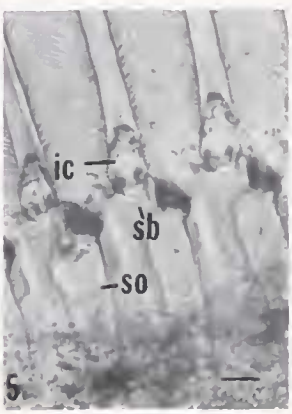
**Figure 4.** Anecdysis, stage $C_2$. Setal organs (so), which give rise to the internal cones (ic), and setal articulations (sa) are evident. (100×), scale bar = 45 μm.

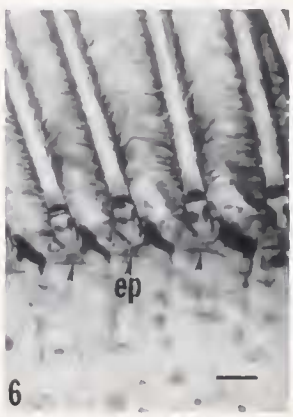
observed for the protein and ecdysteroid titers. The glucose levels were lowest immediately before and after the molt and increased significantly ($P < 0.05$; *t*-test) during anecdysis ($C_3$).

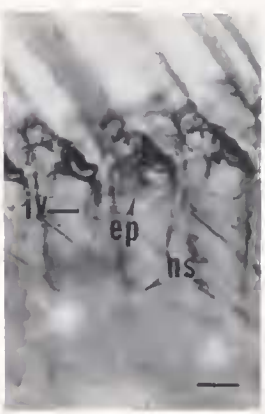
Thirteen proteins were detected in stained native gels; the dominant protein, #6, stained positively for copper with dithiooxamide (data not shown) and is therefore likely to be the respiratory protein, hemocyanin. No distinctive changes in proteins during the molting cycle were revealed by native gels. SDS-PAGE of hemolymph proteins of the molt cycle revealed more than 20 polypeptides (Fig. 14). No changes were observed in the major polypeptides; however, of the less abundant polypeptides, one small (32 kD) and one high molecular weight polypeptide (175 kD) changed in relative abundance during the molting cycle. The relative concentrations of these two polypeptides were low during late metecdysis (B) and anecdysis (C), and increased during proecdysis (D).

## Discussion

The molting cycle in crustaceans is characterized by distinct morphological, physiological, and biochemical events. We have identified and characterized several of these parameters for the South American white shrimp, *P. vannamei*.



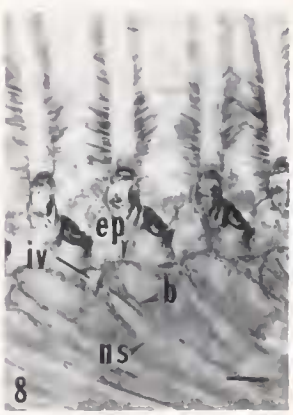




**Figure 5.** Anecdysis, stage $C_3$. Setal bases (sb) have become denser and setal organs (so) more distinct. Well-defined internal cones (ic) are evident. (100×), scale bar = 45 μm.

**Figure 6.** Proecdysis, stage $D_0$. Apolysis. The separation of the epidermis (ep) from the cuticle is evident. (100×), scale bar = 55 μm.

**Figure 7.** Proecdysis, stage $D_1$. Invagination (iv) of the epidermis (ep) has left a clear space between the old cuticle and the epidermis. The new setae (ns) and cuticle have begun to form. (100×), scale bar = 45 μm.

**Figure 8.** Proecdysis, stage $D_1$. The epidermis (ep) has continued to invaginate (iv); new barbules (b) have formed on some new setae (ns). (100×), scale bar = 45 μm.

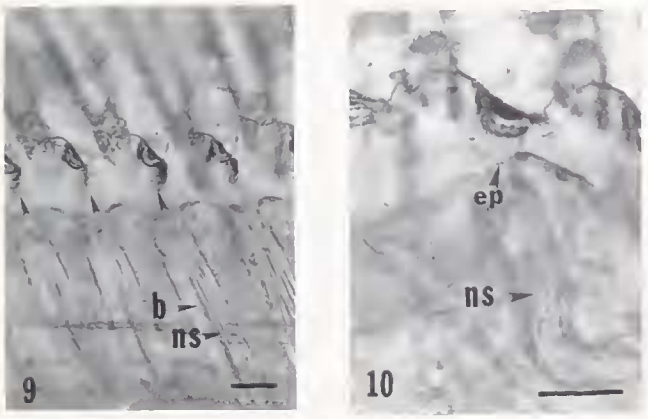



**Figure 9.** Proecdysis, stage $D_2$. New barbules (b) are present on all new setae (ns). (100×), scale bar = 30 μm.

**Figure 10.** Proecdysis, stage $D_3$. The pattern of new setae (ns) is interrupted by folds of the epidermis (ep). The animal is ready to molt in this stage. (120×) scale bar = 40 μm.

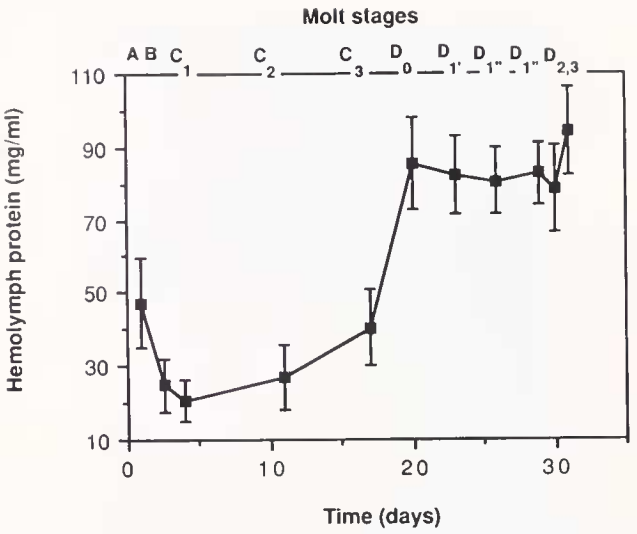



**Figure 12.** Total protein levels in the hemolymph of juvenile *P. vannamei* during the molting cycle. Values represent mean ± S.E.M. (n ≥ 12 for each point).

### *The crustacean molting cycle: setogenesis*

Although setogenesis has been used as a criterion for molt staging for many years (see Drach, 1939), species variations in setal morphology and development result in differences among crustaceans in both staging criteria and in easily-defined subdivision of the molt stages. We have established criteria for the molt stages and substages in *P. vannamei*. These criteria include the discernment in the pleopods of the epidermis, setal lumens, internal cones, and setal organs (see Figs. 1–10). Similar criteria have been used to determine stages for the penaeidae shrimp *P. californiensis, P. stylirostris* (Huner and Colvin, 1979), *P. merguiensis* (Longmuir, 1983), *P. duorarum* (Schafer, 1968) and *P. esculentus* (Smith and Dall, 1985). Setogenesis in these shrimp species differs primarily in the degree of pigmentation in the appendages and in the duration of the molt stages. Furthermore, the complete retraction of the setal matrices was observed in other penaeids during anecdysis, whereas in some individuals of *P. vannamei* retention of setal matrices was observed. Deviations in setogenesis are even more pronounced in other decapods. For example, in the lobster *Panulirus marginatus*, internal cones are lacking; thus,

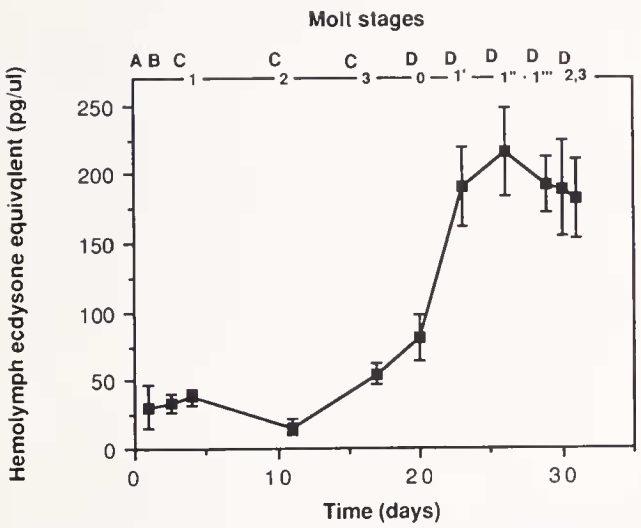



**Figure 11.** Ecdysteroid titers in the hemolymph of juvenile *P. vannamei* during the molting cycle. Each point represents the mean ± standard error of the mean (S.E.M.) (n ≥ 12 for each point).

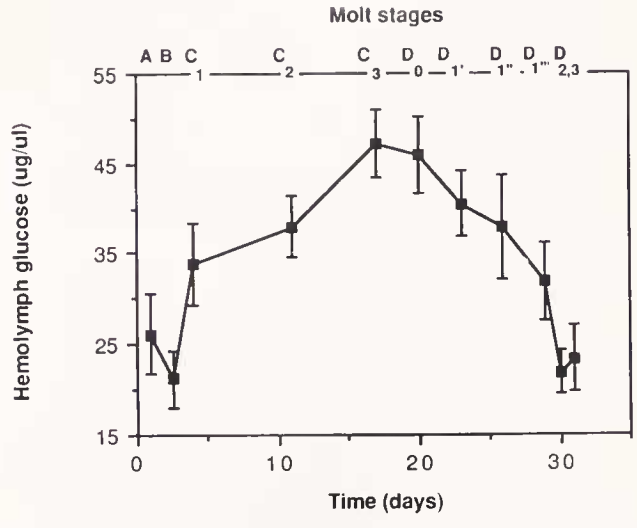



**Figure 13.** Total glucose levels in the hemolymph of juvenile *P. vannamei* during the molting cycle. Values represent mean ± S.E.M. (n ≥ 12 for each point).

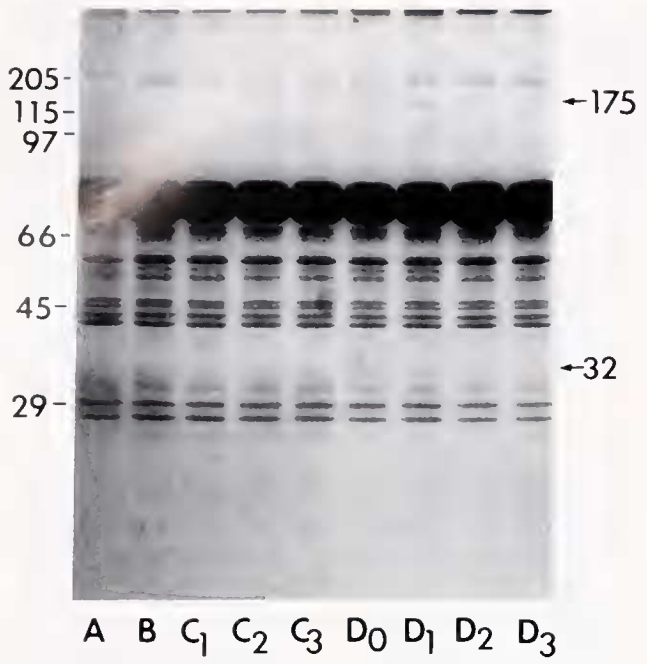



**Figure 14.** 10–15% linear gradient SDS-PAGE of hemolymph proteins from juvenile *P. vannamei* during the molting cycle. Values in the far left column indicate molecular weight determinations (kD). Arrows indicate polypeptides which increase in relative quantities as the molting cycle progresses. Letters (bottom) indicate the molt stage.

the distinction between stages B and C depends mainly on the thin and hollow appearance of the setal lumen in anecdysis (Lyle and MacDonald, 1983). These examples emphasize that molt staging must rely on a combination of setal characters. Furthermore, substaging varies according to the investigators. We found that the molting cycle in *P. vannamei* was readily divided into stages: A, B, $C_{1-3}$, and $D_{0-3}$. In the crayfish *Astacus leptodactylus*, the molting cycle was divided into $A_{1-2}$, $B_{1-2}$, $C_{1-4}$, and $D_{0-4}$ (Van Herp and Bellon-Humbert, 1978). $C_4$ and $D_4$ stages were not described in *P. vannamei* because those putative stages were of extremely short duration.

Molt staging may be accomplished using setogenesis in a variety of appendages. These appendages include the pleopods, as demonstrated in *P. marginatus* (Lyle and MacDonald, 1983), *A. leptodactylus* (Van Herp and Bellon-Humbert, 1978) and *Orchestia cavimana* (Graf, 1972); the maxillae in *Chionoecetes opilio* (Moriysau and Mallet, 1986); and the uropods in *Petrolisthes cinnectines* (Kurup, 1964), *P. stylirostris* (Huner and Colvin, 1979; Robertson *et al.*, 1987) and *P. setiferus* (Robertson *et al.*, 1987). We have used the pleopods for determination of molt stages in *P. vannamei* because removal of other appendages results in trauma or death, and because the relatively thin cuticle of the pleopods facilitates observations on setal development.

Two molting patterns have been defined for the crustacean molting cycle: (1) anecdysic that has a relatively long intermolt and (2) diecdysic that has a long premolt (Knoweles and Carlisle, 1956). In general, crustaceans with an anecdysic molting cycle usually enter a terminal anecdysic ($C_{4T}$) stage (Skinner, 1985). Whether the molting cycle of adult *P. vannamei* (which continue to molt throughout their life) is diecdysic or anaecdysic remains to be determined, but in juvenile *P. vannamei*, the premolt period occupied 50–55% of the molting cycle. Thus, the molting cycle of juvenile *P. vannamei* is diecdysic (Table 1). Our observations on durations of molt stages in juvenile *P. vannamei* are similar to those reported for juvenile *P. esculentus* (Smith and Dall, 1985); however, in juvenile *P. merguiensis* (Longmuir, 1983), juvenile *P. californiensis*, and juvenile *P. stylirostris* (Huner and Colvin, 1979) the $D_0$ stage is proportionally much longer.

### *The crustacean molting cycle: hemolymph ecdysteroids and metabolites*

The titer of hemolymph glucose was low during stages A and B, rose gradually during stage C, reached a maximal concentration in early proecdysis ($D_0$, $D_1$), then declined in late proecdysis (Fig. 11). A similar situation was reported for *Carcinas maenas* (Spindler-Barth, 1976), although titers of circulating glucose in *C. maenas* were more than twice those reported here for *P. vannamei*. In contrast to this pattern, Telford (1968) demonstrated that hemolymph glucose titers increased shortly before ecdysis in three species of crabs. Maximal levels of glucose in *P. vannamei* during the intermolt probably resulted from an accumulation of food reserves during this period of active feeding. Likewise, the gradual decline in glucose titers during late proecdysis corresponded with reduced feeding. We believe that the glucose titers of *P. vannamei* correlate principally with the feeding pattern and do not reflect concurrent changes in metabolism. Since glucose levels were lowest just before and after ecdysis, it is unlikely that the glucose was essential for either chitin synthesis for the new cuticle or as a source of energy during molting. Gwinn and Stevenson (1973) have speculated that in *Orconectes limosus*, the major energy source is chitin because the chitin resorbed by the epidermis before molting provides sufficient material for both new chitin synthesis and energy for molting.

The use of the RIA to measure hemolymph ecdysteroid titers in decapods has been limited to only a few species, including the crayfish, *Orconectes sanborni* (Stevenson *et al.*, 1979) and *O. limosus* (Keller and Schmid, 1979; Jegla *et al.*, 1983), the lobster, *Homarus americanus* (Chang and Bruce, 1980), and the crabs *C. maenas* (Andrieux *et al.*, 1976), *Callinectes sapidus* (Soumoff and Skinner, 1983), *Gecarcinus lateralis* (McCarthy and

Skinner, 1977) *Uca pugilator* (Hopkins, 1983) *Pachygrapsus crassipes* (Chang *et al.*, 1976) and *P. maramoratus* (Charmantier-Daures and DeReggi, 1980). We have shown that in the shrimp, *P. vannamei*, ecdysteroid levels were low during metecdysis and anecdysis, began to rise at apolysis and reached a maximum during proecdysis (Fig. 11). Similar rapid increases in ecdysteroids occur in *C. sapidus* (Soumoff and Skinner, 1983), *O. limosus* (Keller and Schmid, 1979), *G. lateralis* (McCarthy and Skinner, 1977), and *P. crassipes* (Chang *et al.*, 1976). The increasing ecdysteroid titers at the end of anecdysis presumably initiated apolysis. The high ecdysteroid titer was maintained into late proecdysis ($D_3$) but had declined by early metecdysis (A). A similar decrease in ecdysteroid concentrations was reported for *C. sapidus* (Soumoff and Skinner, 1983), *Orchestia cavimana* (Graf and Delbecque, 1986) and *O. sanborni* (Stevenson *et al.*, 1979). The decrease in ecdysteroid concentrations may be in part the result of water uptake to achieve ecdysis. In all reported cases, however, the lowest ecdysteroid titer always occurs during anecdysis (stage C).

Identification of the specific ecdysteroids of *P. vannamei* remains to be determined. In *P. crassipes* (Chang *et al.*, 1976) and *Orconectes* sp. (Carlisle and Connick, 1973), both ecdysone and 20-hydroxyecdysone are found. Only 20-hydroxyecdysone is detected in *O. limosus* (Keller and Schmid, 1979), while both 20-hydroxyecdysone and Ponasterone A are found in *G. lateralis* (McCarthy and Skinner, 1977).

The increase in hemolymph ecdysteroids correlated with an increase in hemolymph protein content (*c.f.* Figs. 11, 12). A comparable pattern was reported for hemolymph protein in *Palaemon serratus* (Baldais *et al.*, 1984), *C. sapidus* (Soumoff and Skinner, 1983), and *O. sanborni* (Stevenson *et al.*, 1979). The increase in hemolymph protein concentration might result from increased protein synthesis (Gorell and Gilbert, 1971), reduced degradation of proteins and/or resorption of cuticular proteins (Travis, 1955).

Hemolymph proteins of several crustacean species have been separated and characterized by gel electrophoresis (Keer, 1969; Fielder *et al.*, 1971). The patterns vary for different species, but hemocyanin is the major protein detected in all cases and accounts for 80–95% of the total hemolymph protein. Since the major protein of juvenile *P. vannamei* stained positively for copper with dithiooximide (data not shown), and was similar in size (74–76 kD) to hemocyanin from *H. americanus* (Senkbell and Wriston, 1980) it is likely that it is hemocyanin. The rest of the proteins may consist of mostly free enzymes (Scheer, 1960). The increase in hemolymph protein observed in *P. vannamei* during the different molt stages (Fig. 12) likely resulted from a general increase in the quantities of the major proteins since no specific changes were observed in the major polypeptides on SDS-PAGE. However, two minor polypeptide subunits, one of low molecular weight (32 kD) and one of high molecular weight (175 kD), increased in relative abundance during proecdysis (Fig. 14). Although minor in quantities, these polypeptides could have important physiological functions, and play pivotal roles in the events of molting. The sources for any of these polypeptides are unknown.

In conclusion, the developmental stage of setae of the pleopods provides a rapid, accurate indication of the molt stage in juvenile *P. vannamei*. Because it is nonsacrificial, repeated measurements may be taken from the same animal to monitor the rate of development. In *P. vannamei*, setogenesis was used to define the molt stages and used, subsequently, to determine molt-related changes in the hemolymph concentrations of ecdysteroids, proteins, and glucose. It is now possible to use carefully staged animals when examining other physiological events such as reproduction.

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